

# TEMPERATURE DEPENDENCE OF BREATHING RATE IN CARP

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Temperature is an essential factor in the aquatic environment; it exerts a profound influence on the morphologic and physiologic characteristics of fishes. To infer from this the importance of temperature in adaptation, in its genetic meaning, seems to be logical.

An adequate study of this adaptation presupposes easily recognizable adaptive variants within a population. Our original intention was to work out an easy method to detect such physiologic variants which would not involve necessarily the death of the experimental objects, for breeding purposes. For several reasons—among which figure the data of Fox (1939) concerning the frequencies of respiratory movements with regard to the geographical distribution of poikilotherms—we have studied the same frequencies in *Cyprinus carpio* L. in relation to temperature. It appeared soon that the breathing rate in this fish is highly dependent on size. As a first contribution to the problem we intended to solve, this paper reports the influence of age and size on the mentioned physiologic trait.

## MATERIAL AND METHODS

Our experiments have been performed on *Cyprinus carpio*, belonging to the race of glass-carps from the temperate warm waters. The best temperature for development for *Cyprinus carpio* would be situated, according to Huet (1953), between 20° and 25° C.; according to Schaeperclaus (1949) the optimum is nearer to 27° C. but at 15° C. a good production is still obtained.

As experimental objects we used fishes of three months, one year, two years and four years old, having live weights from 20 to 2000 grams. The fishes have been obtained from a single commercial stock.

A starvation period of at least 48 hours preceded all experiments.

Each individual of the different weight and age classes has been examined at several temperatures, from 4° C. up until death occurred, at about 38° C. Transfers always took place from lower to higher temperatures. Intervals can be read from the graphs to be discussed presently.

During measurements each temperature was kept constant within 0.1°, and the water abundantly aerated to provide a constant saturation with oxygen.

For practical purposes the fishes were put in small wire baskets. Measurements have been performed only on perfectly quiet animals.

## RESULTS

### *The dependence of the frequency of respiration upon the temperature in a single individual*

#### *1. Temperature acclimation*

By temperature acclimation we mean the temporary adaptive alteration of the phenotype after an external temperature change.<sup>1</sup> It is known from Wells (1935a)

<sup>1</sup>We wish to reserve the term *adaptation* for a similar alteration of the genotype, which is included in the definition of *acclimation* or *acclimatization* by Prosser (1955) and by Bullock (1955).

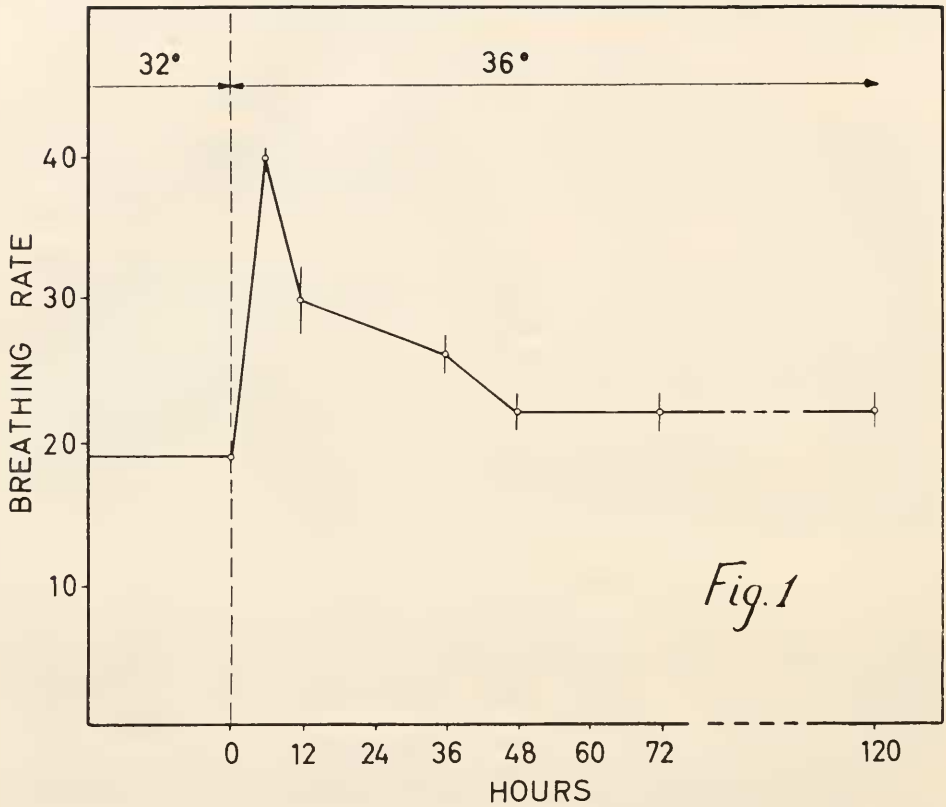


FIGURE 1. Successive mean respiration frequencies after transfer from 32° to 36°, plotted against acclimation time (carp No. 16). Vertical bars added to the points cover the range  $M \pm \sigma$ .

TABLE I  
*Frequency of respiration as a function of temperature*  
(Carp No. II)

Temperature (° C.)	Duration of respiratory pauses	Number of pauses per minute	Breathing frequency
4	1-2 mins.	1	2
8	$\frac{1}{2}$ -2 mins.	2-1	6
12	15 secs.	3	9
18	—	—	24
15	16 secs.	3	13
20	10 secs.	5	24
26	10 secs.	5	25
29	10 secs.	5	24
34	10 secs.	5	24
35	—	—	24
37.4	3 secs.	15	32

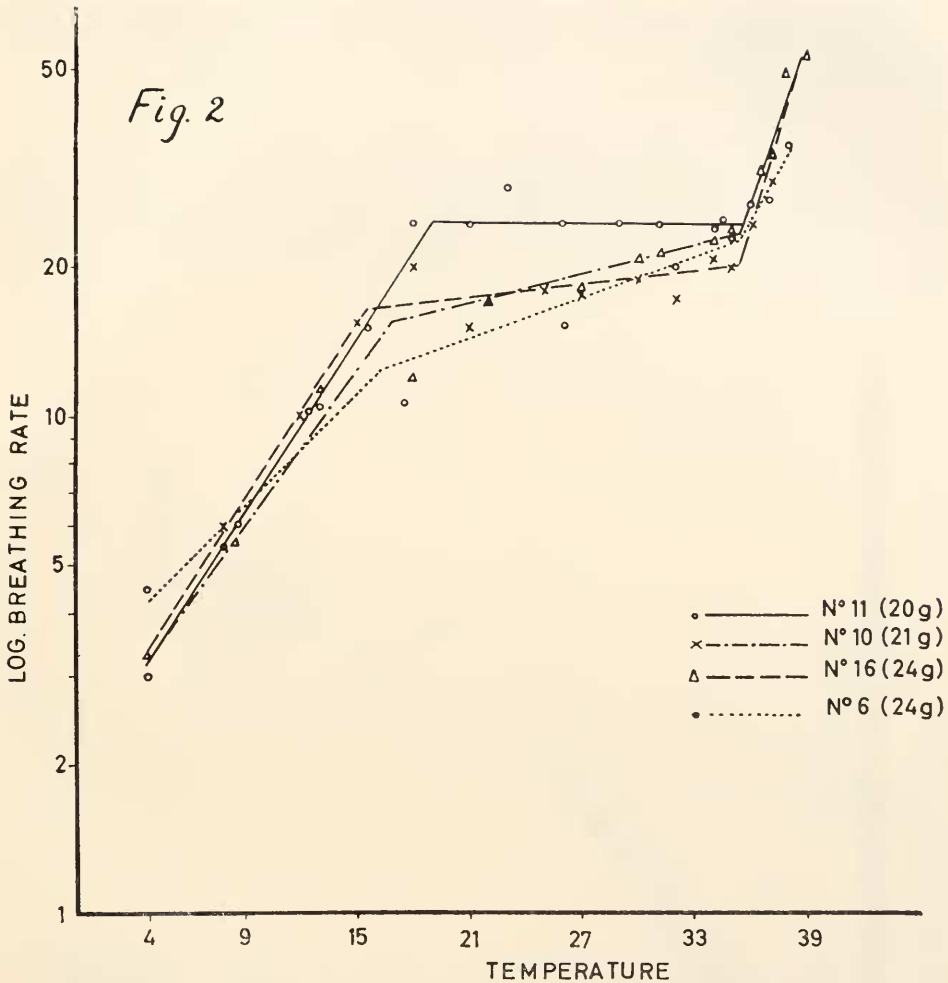


FIGURE 2. Rate-temperature curves for breathing frequency in carps of 20 to 24 grams.

and Schlieper (1950), that, in fishes, this acclimation takes from 24 hours to a few days after the transfer from one temperature to another. Only after this lapse of time, constant and consistent results, on metabolism at least, have been obtained. Before acclimation is completed, the oxygen consumption is generally higher than normal.

Figure 1 shows that similar phenomena characterize the accommodation of the frequency of respiratory movements. This frequency, after an initial rise of 100%, reaches a stable value after 48 hours following a transfer from 32° to 36° C. Usually a steady-state is reached within three to four days, although rarely it may last fourteen days. Acclimation is generally shortest between 16° and 30°. All fishes which maintained this steady-state for 48 hours were considered as completely acclimated. Only measurements obtained on such fishes are considered further in this paper.

At given high temperatures the fishes die before having attained complete acclimation as defined. These temperatures are indicated in this paper as the upper lethal temperatures.

2. *Respiratory pauses and frequency of respiration at a given temperature*

Typical for the respiration of fishes, especially of small ones, are the so-called pauses, during which no respiratory movements occur. After such a pause, which may be variable in length, the fish breathes a few times at a rapid rate and then pauses again. The duration and the frequency of the pauses vary with the temperature (Table I).

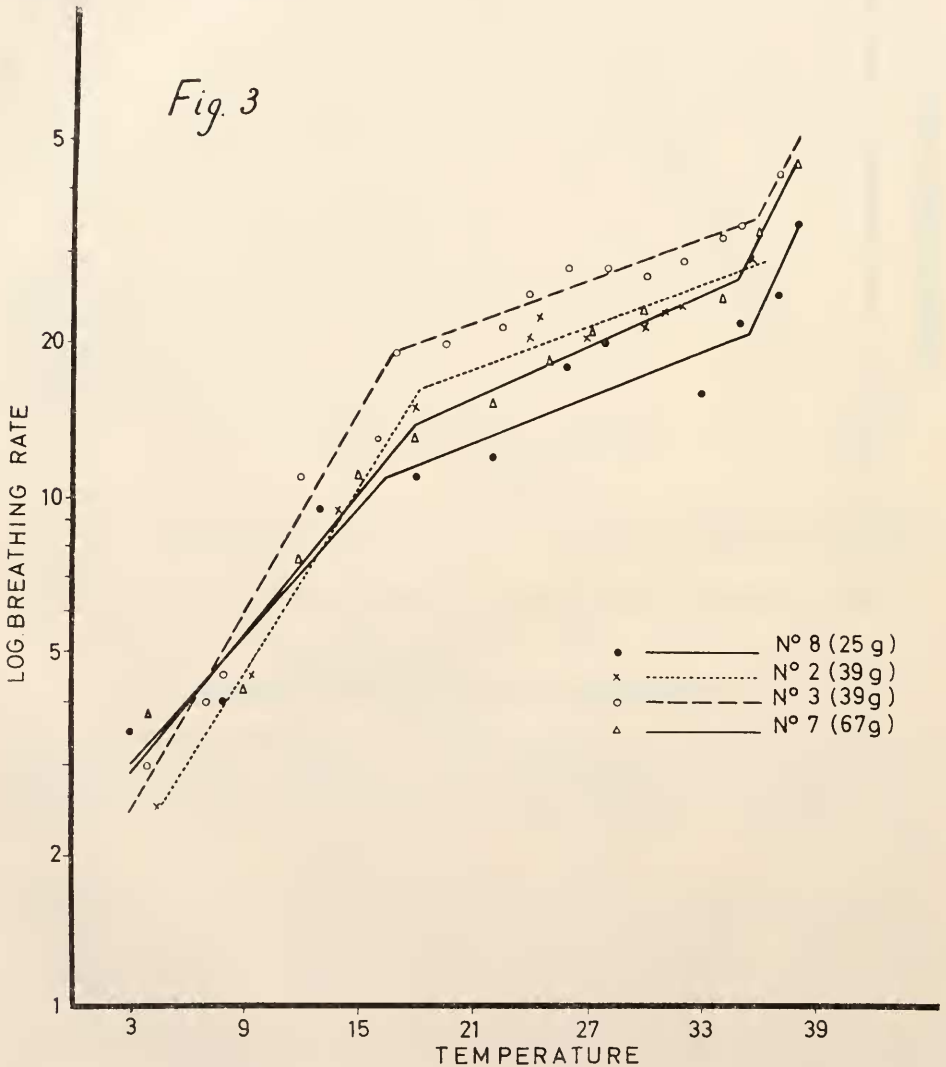


FIGURE 3. Rate-temperature curves for breathing frequency in carps of 25 to 67 grams.

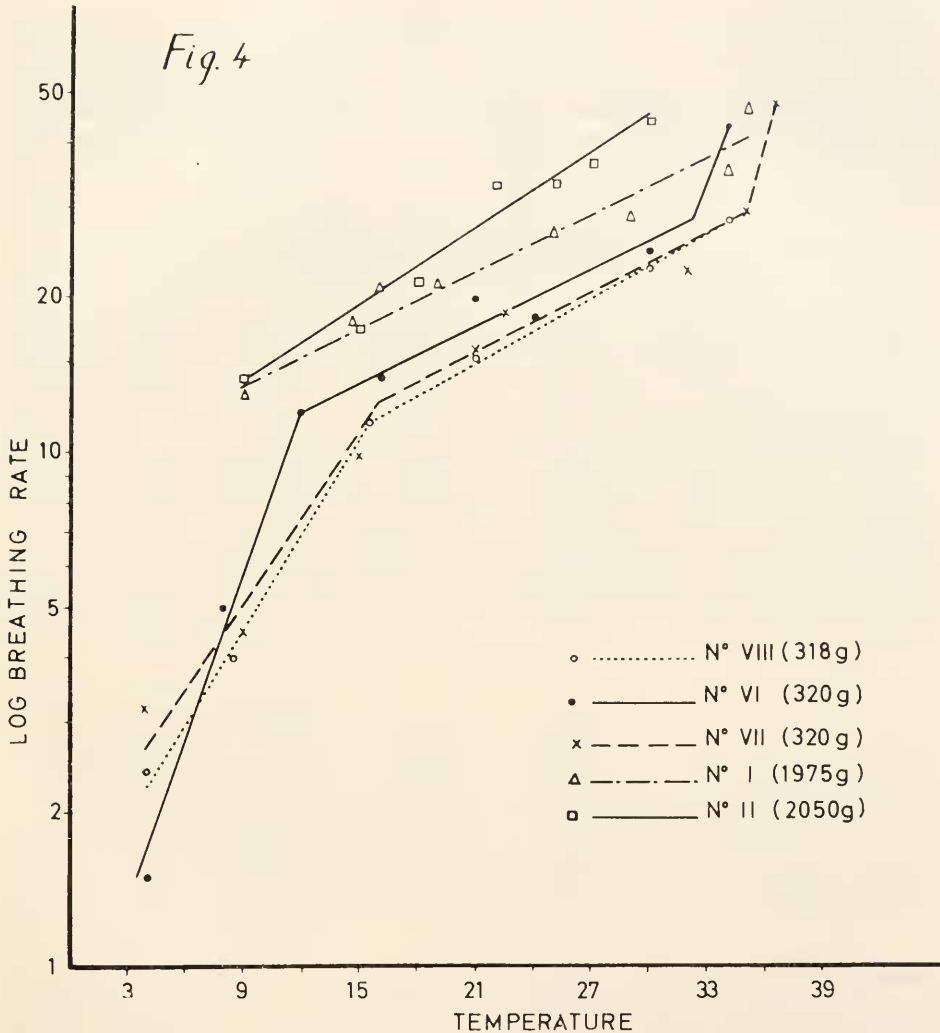


FIGURE 4. Rate-temperature curves for breathing frequency in carps of 318 to 2050 grams.

In this paper breathing frequency is expressed as movements per minute, ignoring the pauses. The time intervals used for one count were chosen long enough to eliminate the disturbing influence of the pauses. As a rule these intervals were five minutes at low, and two minutes at high temperatures, the counts being repeated at each temperature as many times as needed for adequate statistical treatment. Immediately after transfer to a higher temperature no respiratory pauses may occur, making for lower standard deviations (Fig. 1). Otherwise no trends in standard deviations could be detected during acclimation, with respect to time, or after acclimation, with respect to temperature.

3. *Respiration frequency as a function of temperature*

The variations of the mean frequencies of respiratory movements with respect to temperature, show, in the case of the smaller fishes, a typical pattern: first, at lower temperatures, a progressive increase of the frequency with a rise in temperature; further a less steep or even horizontal central part, where the frequency in-

TABLE II  
*Q<sub>10</sub>-values,\* breathing rates at 24° and lethal temperatures for carps,  
arranged according to weight and age*

Serial number	Weight in grams	Age in years	Q <sub>10</sub>			Rate at 24°	Lethal temperature
			Low	Intermediate	High		
11	20	$\frac{1}{3}$	2.10 (4-19)	1.00 (19-35.4)	2.82 (35.4-37)	24.6	38
10	21	$\frac{1}{3}$	2.13 (4-15.6)	1.10 (15.6-35.4)	3.84 (35.4-38.4)	18.0	38.5
6	24	$\frac{1}{3}$	1.98 (4-17)	1.22 (17-35.2)	3.36 (35.2-38)	18.2	39
16	24	$\frac{1}{3}$	1.69 (4-16.3)	1.30 (16.3-35.3)	2.28 (35.3-37.8)	15.9	38
8	25	$\frac{1}{3}$	1.84 (3-16.5)	1.31 (16.5-35.4)	2.31 (35.4-37.5)	14.2	38
2	39	$\frac{1}{2}$	2.07 (4.7-18.3)	1.26 (18.3-35)	—	19.5	39
3	39	$\frac{1}{2}$	2.16 (4.7-17)	1.31 (17-35.5)	2.66 (35.5-37)	14.0	39
7	67	2	1.89 (4-18)	1.37 (18-35)	2.66 (35-37.7)	17.6	38
VIII	318	2	2.19 (4-15.5)	1.40 (15.5-34)	—	17.4	36
VI	320	2	2.83 (3.5-12.3)	1.35 (12.3-32)	—	20.2	36
VII	320	2	2.08 (4-16)	1.34 (16-35)	4.20 (35-36.5)	18.2	37
IX	325	2	—	—	—	—	35.8
I	1975	4	—	1.39 (9-35)	—	25.9	36
II	2050	4	—	1.51 (9-30)	—	33.0	36

\* The temperature limits, in degrees C., are indicated in brackets.

creases much less with temperature, and finally a sudden rise of the respiratory rate at a point which is near to the lethal temperature.

This pattern is particularly well recognizable when the data are plotted on semilog coordinates, showing the proportionate response of the breathing rate to the temperature (Figs. 2, 3 and 4). This proportionate response appears to show, over broad temperature ranges, practically constant values, which change abruptly to other such values, at given critical temperatures. From eye-fitted curves through the data for individual fishes  $Q_{10}$ -values for each of these ranges can be readily estimated, while their intersections localize, with a fair approximation, the critical temperatures. The lower critical temperatures appear to be situated between  $12.3^\circ$  and  $19^\circ$ , the higher between  $32^\circ$  and  $34.5^\circ$  (Table II). The lower critical temperatures delimit a first zone of high  $Q_{10}$ -values from 1.69 to 2.83. Within the range extending from the low to the high critical temperatures  $Q_{10}$ -values vary from 1.00 to 1.51. Within the range of high temperatures  $Q_{10}$ 's reach values between 2.28 and 4.20. Because of the short interval separating the high critical and the lethal temperature, only a few data can be obtained in this range, making for a limited significance of these last  $Q_{10}$ 's for most fishes.

Nevertheless it is perfectly clear that for the population of carps studied, typical regulation phenomena characterize the respiratory rates over given homeostatic zones between  $12.3^\circ$  and  $34.5^\circ$ . However, within these zones a considerable individual variation, as to the  $Q_{10}$ 's, as well as to the situation of the critical temperatures, is showing up.

#### a. The influence of weight

A closer inspection of the log R-T curves in connection with the weight variants, leads readily to the recognition of the following facts.

First, all small fishes (39 grams and lower) exhibit more effective regulatory mechanisms than larger fishes. In the first category all  $Q_{10}$ 's, within the homeostatic zone, fall between 1.00 and 1.31, and, moreover, the correlation between weight and the respective values is very close (Table II). All larger fishes, on the other hand, have  $Q_{10}$ 's between 1.34 and 1.51 and, again, the largest fishes have very low regulatory capacities.

Concomitant with the occurrence of lower  $Q_{10}$ 's within the homeostatic zones of larger fishes, is the lowering of the upper lethal temperature threshold of the same fishes, with respect to the small fishes. Fishes of 67 grams and less have lethal temperatures at  $38^\circ$  and  $39^\circ$ , while those of higher weights have lethal temperatures between  $35.5^\circ$  and  $37^\circ$ . On the other hand, the largest fishes ( $\pm 2000$  grams) fall easily into a lethargic state at  $4^\circ$ , where the measurement of barely recognizable respiratory movements is no more possible. Therefore, a subdivision of the R-T curves of large fishes into three, with differential regulatory characteristics, seems to be impossible. Their narrowed viable range of temperatures can be characterized only by a single, high  $Q_{10}$ -value.

A last remarkable feature is the variation of the mean respiratory rate. The different individual R-T curves are clearly situated at unequal over-all rate levels. It is difficult to express this shift of the curves along the ordinates. We have tried to do this by indicating the estimated rate for all fishes at  $24^\circ$  (Table II). It appears, then, that the largest fishes have very high rates, smaller fishes attaining



only about half of these rates. The smallest fish, however, shows again a high over-all rate.

b. The influence of age

On the basis of our data it is not possible to differentiate clearly the effect of age from the influence of size. A certain indication is obtained from fish No. 7 (Table II). This fish was intentionally starved during one year in the laboratory. During that year its weight practically remained constant (67 grams), and was far below the weight of normally fed fishes of the same age ( $\pm 320$  grams). For as far as the lethal temperature is concerned ( $38^\circ$ ) this fish reacted according to its weight class. Its  $Q_{10}$  within the homeostatic zone, however, falls within the range of its age group (1.37).

c. Residual variation

The number of individuals studied within each weight and age class does, of course, not allow an estimation of the individual variation of breathing rate within a homogeneous group. From our data we can, however, infer its existence. Most interesting, in this respect, is the fact that the R-T curve of fish No. VI is, in its whole, shifted to lower temperatures, for a distance of about  $3^\circ$ , when compared to fish No. VII, belonging to the same weight and age group (Fig. 4 and Table II). This might indicate a differential adaptation to temperature of both fishes.

Individual variation, with respect to the efficiency of the breathing regulation within homeostatic zones of equal extension, seems to exist as well. This is indicated by the detected differences in  $Q_{10}$ 's within age and weight classes.

## DISCUSSION

Two main points of interest are revealed by our analysis. The first is the shift of the upper lethal temperatures from  $38^\circ$ – $39^\circ$  for small fishes, to  $35$ – $36^\circ$  C. for large-sized individuals. The second is the gradual disappearance of homeostasis for breathing frequencies with increase in size.

Decreased resistance to high temperature with size, if we restrict the survey to post-embryonic life, is not a general trend among fishes. The literature concerning the subject is reviewed by Hart (1952). All three types of fish species seem to exist: those with increasing, those with stationary, and those with decreasing resistance with increasing size. In most cases the separate effects of size and age have not been studied. In *Salvelinus fontinalis* no size effect was recorded for yearlings of the same age (Fry *et al.*, 1946), indicating seemingly that the age effect on temperature resistance would be preponderant. However, Spaas (unpublished results) found a size effect in one-year-old trout and a mean increase in high temperature resistance in two- and three-year-old fishes of the same species, which, however was lower than expected on the basis of the size differences for temperature tolerance within yearlings. Both factors, age and size, seem to interact in some way. The few data recorded in this study are not opposed to such a view.

A second main point, disclosed by our analysis, is the progressive increase of dependence of the frequency of respiratory movements upon the temperature. It



is illustrated by the gradual disappearance of a homeostatic zone of practical stability of this frequency at several temperatures in small fishes, and by a correlated increase of the over-all  $Q_{10}$ -value of this process in the course of life history.

By reputation, poikilotherms are animals without homeostatic mechanisms regulating their most essential physiologic functions, such as oxygen consumption, heat production, activity and development. More specifically, for oxygen consumption, they have been generally assumed to follow Krogh's "Normalkurve."

However, a certain number of workers have shown that the type of response to temperature of such functions is not only a specific trait among poikilotherms, but even that it varies intraspecifically as well.

According to recent reviews of the data (Rao and Bullock, 1954; Bullock, 1955), it seems justified to admit that in most cases a higher dependence on temperature is characteristic for animals from warmer habitats. A survey of the data on speed of development leads to the same conclusion with regard to specific and to racial geographic variation (Heuts, 1956). More effective homeostatic mechanisms seem to be a distinctive trait of inhabitants of colder areas, especially when intraspecific comparisons are made. Poikilotherms are poikilostatic organisms, but they are such to varying, genetically determined, degrees.

On the other hand, it is clear enough that the homeostatic mechanisms of the homoiotherms also are operating only within a given temperature range in a given individual (the thermoneutral zone), and, further, that the ranges of this zone are equally subject to specific variation in relation to the habitat, northern animals possessing more efficient homeostatic mechanisms operating at low temperatures (Scholander *et al.*, 1950).

The conclusion is obvious, and has been drawn, that a homeotherm is clearly adapted to the temperature range where the  $Q_{10}$  for biological processes appears to be equal to 1.00, and where, consequently, its behavior is most homeothermic.

In poikilotherms such homeostatic zones, with  $Q_{10}$ 's approaching 1.00, are generally not found or not recognized.

Only recently Bullock (1955) strongly called attention to the existence of homeostatic mechanisms for a number of rate processes among several poikilotherms. In addition to the cases cited by this author a few other examples may be mentioned here.

A clearly delimited homeostatic zone for oxygen consumption is indicated, although not recognized by the author, in *Fundulus parvipinnis* between 18° and 22° for small fishes (Wells, 1935a, 1935b). Its existence shows up due to almost continuously increasing experimental temperatures between 10° and 24°. A definite fall of  $Q_{10}$  values for oxygen consumption between 15° and 20° in *Salvelinus fontinalis* is demonstrated by Job's data (1955). Unfortunately no measurements have been made beyond 20°. The same author mentions the results of Edwards (1946) on the click beetle *Melanotus communis*, which, between 17° and 27°, shows a definite decrease of  $Q_{10}$ -values for oxygen uptake. Job, however, is inclined to regard this phenomenon as due to an experimental error.

A number of indications seem thus to point to a possibly widespread phenomenon of homeostatic mechanisms among poikilotherms, which may be brought to light by more accurate experimental procedures. These zones being presumably narrow, their absence in current observations might be due to the experimental

procedure making use of too discontinuous temperature gradients. Still another source of error might be an insufficiently long acclimation to the experimental temperatures.

If, however, homeostatic zones can be demonstrated to exist in poikilotherms, then it seems logical to admit, as is done in homeotherms, that they delimit adaptive zones, and to conclude that a poikilotherm is adapted to such conditions, where it is least poikilostatic. Such a situation exists clearly in young carps. In the smallest fishes  $Q_{10}$ -values approach 1.00 over a temperature range from 16° to 35°. Within this range they are homeostatic for the studied trait, and to this range they are probably best adapted.

How far the frequencies of respiratory movements reflect the oxygen consumptions at different temperatures remains, of course, an open question. They will not truly reflect oxygen consumption, if, principally, the utilization coefficient of the oxygen present in the water, flowing over the gills during each breathing movement, would be different according to temperature. For trout and eel, at least, van Dam (1938) has shown that the utilization percentage is not affected by a temperature difference of 8°.

For obvious reasons, the complete disappearance of homeostatic zones in carps, with increasing size, cannot be asserted. They certainly are narrowed in the course of life, though this progressive alteration is not, or not directly, dependent on age. A similar narrowing of homeostatic zones is obvious in *Fundulus parvipinnis* (Wells, 1935b), concomitant with the over-all higher dependence of oxygen uptake on temperature with increasing size.

In other cases, however, the individual evolution is exactly the opposite. Job (1955) recognizes a flattened proportionate response to temperature, a higher temperature independence, in large-sized *Salvelinus fontinalis*. Similarly Spaas (unpublished results) finds an increased dependence of oxygen consumption in large versus small yearlings of brook- and sea-trout.

The click beetles already mentioned (Edwards, 1946) show, on the other hand, a response of oxygen uptake to temperature per unit wet weight, which is independent of size. The case is, however, not strictly comparable, because all click beetles, whatever their dimensions, have reached final sizes.

Between the first fact disclosed by our analysis (the shift of upper lethal temperature to lower values in the course of life) and the second (the decrease in homeostatic efficiency) there seems to be a close relation in our material, as one would logically expect. Lack of adequate data on post-embryonic development prevents the generalization of this observation for individual life cycles. Some data on embryonic development, however, indicate clearly that a similar relationship does not hold in racial and interspecific comparisons.

Races or species with a higher temperature independence for speed of embryonic development can nevertheless have a narrower temperature tolerance range during development than another race or species with a more dependent development. If the relationship between temperature tolerance limits and regulation holds in individual cycles, then this cycle can be labelled as physiologically regressive (with respect to the general trend of the phylogenetic record) in *Cyprinus carpio* and in *Fundulus parvipinnis*. Several Salmonidae seem to follow a physiologically progressive ontogeny.

A last point of interest is the fact that the methods used seem to be adequate to

detect individual adaptive differences to temperature ranges, as well as individual differential degrees of homeostatic attributes, without necessarily killing the experimental objects. This permits genetic studies of the mentioned characteristics, and creates thus the possibility of filling gradually the evolutionary gap between poikilotherms and homeotherms.

## SUMMARY

1. The dependence of the frequencies of respiratory movements upon temperature has been studied in *Cyprinus carpio* L.

2. The degree of dependence of these frequencies upon temperatures is primarily determined by the size of the fishes. Large fishes are highly, small fishes only slightly, dependent.

3. Small fishes are characterized by a broad homeostatic zone of independence of the breathing frequency on temperatures. This homeostatic zone disappears in large fishes. The upper lethal temperatures decrease concomitantly with the disappearance of the homeostatic zones.

4. Individual variants, as to the degree of homeostasis and as to the adaptive temperature ranges, can be detected by the method presented. It can be used for studying genetic determination of these characteristics.

5. A survey of the data in the literature allows one to distinguish poikilotherms with progressive and with regressive ontogenies, with respect to homeostatic behavior.

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